PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Randomized controlled trial of vitamin D supplementation in sarcoidosis
AUTHORS	Bolland, Mark; Wilsher, Margaret; Grey, Andrew; Horne, Anne; Fenwick, Sheryl; Gamble, Greg; Reid, Ian

VERSION 1 - REVIEW

REVIEWER	Jake Olivier Senior Lecturer School of Mathematics and Statistics University of New South Wales Sydney Australia I have no competing interests to declare.
REVIEW RETURNED	28-Jul-2013

THE STUDY	Since the observation period is over a year, it is common for patients to be lost to follow up (although complete data is not impossible). Would the authors care to elaborate? This is not clear in the manuscript. For the statistical analysis, a mixed model is appropriate here but could the authors give more information regarding how the within-subject variability was modeled? Was is unstructured, for example? Were p-values adjusted for multiple comparisons for Tukey post-hoc comparisons? It would be more appropriate to say the level of significance was set at alpha=0.05. I guess you could say hypothesis tests were deemed significant for $p<0.05$. It's not quite correct as it is. A CONSORT checklist would be beneficial to the article's presentation.
RESULTS & CONCLUSIONS	The authors state multiple times that "vitamin D supplementation had NO EFFECT on..." Given such a small sample size, this would appear to be a stretch. It would be more appropriate to say the results were similar between the groups. The figure numbers don't match up in the text. Figure 1 on page 10 points to Figure 2, I believe. On page 14, line 52, it is unclear how post-hoc power was computed and it is also unclear how this is relevant considering all non-significant comparisons suffer from low power (i.e., anything is significant with a large enough sample size).
REPORTING & ETHICS	a CONSORT checklist was not given and its inclusion would make this more clear.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Comments to the Author
This manuscript reports results of a trial of vitamin D supplementation conducted in 27 patients with sarcoidosis, recruited over a 3-year period in New Zealand. No benefit of the intervention was shown in terms of bone mineral density, and supplementation precipitated hypercalcaemia in one participant in the intervention arm of the study.
Major comments
1. The trial is very small – n=27 – and did not achieve its modest target sample size (n=40). The negative results on BMD therefore carry little weight, as they are likely to have arisen due to type 2 error. The main conclusion of the paper – 'an absence of benefit from vitamin D supplements' is therefore not supported.

Response:
As noted under Statistical reviewer point 7, trials in which results that are not statistically significant may occur because of lack of power (a Type II error) or lack of treatment effect. We acknowledge in the limitations section of the Discussion, the study is small. Nevertheless, it was large enough to detect clinically relevant effects on surrogate measures of skeletal health. Decreases in bone turnover markers of 20% for P1NP and 0.5% for total body bone density would have been statistically significant in a study of this size. Therefore, we think the conclusion that the vitamin D supplement did not benefit surrogate markers of skeletal health in this study is appropriate. If vitamin D does have benefits (ie the results are a Type II error), these are likely to be very small and of uncertain clinical relevance.

2. Generalisability of results is compromised by the exclusion criteria that were employed. Patients with BMD T score <2.5 were excluded. This is the very group that might be expected to derive the most benefit from vitamin D in terms of bone density. Excluding these patients significantly reduces the generalisability of the study.

Response:
Including high risk patients in placebo-controlled trials can have important ethical considerations, and so patients at intermediate risk are often included in such trials instead. Consequently, many trials in the osteoporosis field have been carried out in patients with osteopenia. Generally, the results from trials in osteopenic populations for surrogate markers like bone turnover and bone density have been very similar to the results from trials in osteoporotic populations. Where results on surrogate endpoints in intermediate populations are positive, this provides a rationale to do large trials with hard endpoints in higher risk groups. We therefore think the trial results are relevant for individuals with sarcoidosis and low vitamin D levels.

3. It's also not clear why patients with 25D levels of 50 – 75 nmol/L were excluded. There is plenty of observational evidence to suggest that their BMD might also benefit if brought into the optimal range (>75 nmol/L).

Response:
We restricted the trial to people with lowest 25OHD levels who are most likely to benefit from vitamin D supplements. We think it is extremely unlikely that including patients with higher 25OHD levels who are less likely to benefit from vitamin D supplements would alter these results. We have a recently conducted a meta-analysis of randomised controlled trials which demonstrates that vitamin D

supplementation in individuals without sarcoidosis does not increase bone density when baseline 25OHD levels are >50 nmol/L (Reid et al, Lancet, in press).

3. Novelty is limited: don't we already know that vitamin D supplementation may precipitate hypercalcaemia in patients with sarcoidosis?

Response:
We are not aware that any randomised controlled trial of vitamin D supplements in sarcoidosis with the endpoints we studied has ever been carried out.

Minor comments
4. Abstract:
Methods – what does the term '1y' add here?

Response:
As suggested, we have deleted this term.

5. Results – abstract should present what difference in 1,25 levels was between groups at 4 weeks – current wording is uninformative The abstract and summary should make it explicit that this trial was conducted in patients with baseline normocalcaemia

Response:
We have not included the absolute 1,25OHD levels because of word limit constraints. We have added text regarding normocalcaemia to the abstract and summary as suggested.

6. Introduction: 'Furthermore, countries at higher latitudes do not have consistently lower prevalence of hypercalcaemia than countries closer to the equator,[1] and prevalence of hypercalcaemia is similar in countries with and without dietary vitamin D fortification.[6] The relevance of this statement to the subject of the paper – sarcoidosis – is not apparent to this reviewer

Response:
We have clarified that both these statements refer to the prevalence of hypercalcaemia in sarcoidosis. The relevance of the statements is that if 25OHD contributes to hypercalcaemia in sarcoidoisis, countries with low sunlight exposure or without dietary vitamin D fortification would be expected to have lower average 25OHD levels and therefore lower prevalence of hypercalcaemia in sarcoidosis.

7. Methods Why were two different 25(OH)D assays used to measure vitamin D status in the trial?

Response:
We screened participants using a locally available 25OHD radioimmunoassay, then analysed all the study samples in a single batch at a remote laboratory using a liquid chromatography tandem mass spectrometry assay, which is generally considered the most accurate assay for 25OHD.

8. Statistics – it's unconventional to compare baseline characteristics of intervention vs. control groups in a clinical trial with statistical tests (Table 1) – see Consort statement

Response:
As suggested, we have removed the P-values from this Table

9. If BMD data were analysed raw, then raw data should be presented

Response:
It is standard practice in trials of bone density to report the data using percent change from baseline for ease of interpretation, but to analyse the data using raw data which have greater statistical power. Because absolute bone density values are different at each skeletal site and for each make of bone densitometer, absolute values are not informative for most readers.

10. P11 line 45 should read Figure 2

Response:
We have corrected this error.

11. Discussion
The authors have cherry-picked negative meta-analyses to cite (p14 lines 3-5) – there are plenty of positive ones that should also be cited for balance.

Response:
Our statement is accurate- that meta-analyses of vitamin D supplements (in the absence of calcium co-administration) have not shown benefits on the stated outcomes. We did not cherry pick negative meta-analyses. Instead we have reported the latest meta-analyses on these topics that reported the effects of trials of vitamin D supplements without calcium. We are not aware of any meta-analyses of trials of vitamin D supplements used without calcium that show benefit on falls, fractures, cancer or cardiovascular events.

12. The authors appear to have conducted retrospective power calculations to determine the effect sizes that their power could have detected – this is methodologically suspect – see Hoenig JM, Heisey DM. American Statistician. 2001;55:19-24; Goodman SN, Berlin JA. Ann Intern Med. 1994 Aug 1;121(3):200-6.

Response:
We have addressed this point in detail under Statistical Reviewer, point 7.

Reviewer: 2
Comments to the Author
This paper describes a 1 year placebo controlled, double-blinded, randomised controlled trial of vitamin D supplementation in patients with mild vitamin D deficiency and sarcoidosis. The study was powered to detect a change in serum calcium as its primary endpoint requiring 40 patients recruited to the study to do so. Secondary end points of urinary calcium excretion and measures of bone mineral density (BMD) and markers of bone turnover were also considered.
In considering this paper I have taken advice from colleagues in the department of metabolic bone disease.

1.The use of serum calcium as a primary endpoint is questionable. We feel that it is unlikely to be a

robust endpoint and in the patient group described we would not expect changes in this parameter. The use of high doses of Vit D supplementation in very deficient patients does, in routine clinical practice, result in some changes in serum calcium but even this is usually relatively minor and in no way reflects the underlying bone effects of Vit D supplementation.

Response:
We are uncertain why the Reviewer suggests that serum calcium is a questionable endpoint. As we state in the Introduction, hypercalcaemia following vitamin D supplements in sarcoidosis is described, and recommendations exist to avoid use of vitamin D supplementation in patients with sarcoidosis because of concerns about hypercalcaemia. However, no clinical trial has addressed this issue. We chose serum calcium as the primary endpoint because of concern that vitamin D supplements may frequently cause hypercalcaemia, and thus safety is a primary concern in prescribing these supplements in sarcoidosis. We agree with the other comments about vitamin D supplements in the general population but do not think they are relevant to this study which was restricted to patients with sarcoidosis.

2. Furthermore although the study was powered to require 40 patients only 26 completed the study.

Response:
We have discussed this issue in detail under point 7, statistical reviewer. We acknowledge this as a limitation to the study, but pointed out in the Discussion, that a difference in 0.06 mmol/L for serum calcium between the groups would have reached statistical significance. Our pre-study power calculation suggested 40 people would have been required to have 80% power to detect a 0.1 mmol/L difference in serum calcium which we thought would be of clinical relevance. Therefore, the sample size was adequate to detect greater differences in the primary endpoint than was assumed for our a priori power calculation.

3. The urinary calcium excretion also appears high in the population – it would be expected that, in the face of clinically relevant vitamin D deficiency, that urinary calcium excretion would be reduced.

Response:
The definition of vitamin D insufficiency/deficiency is controversial. Reviewer 1, for example, considers 25OHD level <75nmol/L to be indicative of insufficiency/deficiency (Point 3, Reviewer 1). The participants all have 25OHD measurements <50 nmol/L which is widely accepted as indicating vitamin D insufficiency. 25OHD is considered the best marker of vitamin D status whereas urinary calcium excretion is not used to diagnose vitamin D deficiency. Not all people with 25OHD levels <50nmol/L have secondary hyperparathyroidism, so will not necessarily have low urinary calcium excretion. Urinary calcium excretion is also known to be elevated commonly in sarcoidosis.

4.The use of BMD as a marker is also felt to be of limited use in that the timeframe is too short to demonstrate significant changes.

Response:
We disagree. Numerous studies have been conducted with BMD as an endpoint over 12 months and many agents show statistically significant effects in studies over this time frame.

5.Although the literature is conflicted in this area the authors suggest that the management of patients with sarcoidosis using dietary restriction and sunlight avoidance could promote Vit D deficiency and

lead to long term effects on bone health. However there seems little to support this statement in terms of demographic evidence and I am concerned that the authors are addressing a clinical question of no real relevance.

Response
The association between low vitamin D status and low bone density is well described (for example Bischoff-Ferrari HA., Am J Med 2004;116:634-9). As described in the Introduction, several cross-sectional studies have shown high prevalence of low bone density in sarcoidosis, and recommendations exist to avoid vitamin D supplementation in sarcoidosis. Thus, the hypothesis that low vitamin D status might be associated with low bone density in sarcoidosis, which might improve by repletion of vitamin D, is reasonable and consistent with existing evidence.

6. Methodologically there are concerns. The patient group described are predominantly middle aged women. As the hormonal changes of the menopause can dramatically influence calcium and bone physiology the study should be limited to those who are either premenopausal or have been post menopausal for at least 5 years. There is no evidence that this has been controlled for or checked/confirmed.

Response:
There were similar numbers of women in each treatment group and similar age distributions between the groups. In each treatment group only 2 women were premenopausal. Therefore we think this is unlikely to have altered the study results.

7. Furthermore the dietary calcium intake has very high standard deviation suggesting a very variable intake amongst participants – a further substantial confounder.

Response:
The high dietary calcium intake standard deviation in the vitamin D group was caused by one woman having a very high calcium intake (~2660 mg/day). We think this is unlikely to have caused substantial confounding since there is little evidence that dietary calcium intake impacts on BMD (Anderson JJ, J Clin Endocrinol Metab 2012;97:4531-9.).

8.There are also methodological concerns regarding the use of different assay methods that are described by the authors. P1NP is a marker of bone formation. Its levels are reduced in a dose dependent way with steroids, in response to fractures, with surgery and in renal or liver disease/impairment. There is insufficient detail provided regarding these parameters, including inhaled steroid doses and urinary cortisol excretion, for example, to believe the groups are well matched.

B-CTX is a marker of bone resorption. While this is less influenced by steroid therapy or fracture samples must be taken in a consistent manner – the samples should be fasting samples, taken at the same time of the day in all individuals. There is insufficient explanatory information to know whether these quality markers have been maintained.

Response:
The text states the all samples were taken fasting and in the morning. We did not collect information on urinary cortisol. The bone turnover markers were similar in each group at baseline and remained very stable in the placebo group throughout the trial. While we cannot rule out differential baseline differences because of factors the Reviewer raises, we think these are unlikely to have substantially

altered the findings. We are not aware of evidence that usual doses of inhaled glucocorticoids or variation in daily cortisol production within the normal range affect bone turnover or bone density. As stated in the text, only 1 participant took prolonged oral glucocorticoids during the study.

9. Finally the use of parametric statistical tests is questioned as in sample sizes of only 13-14 patients reassurance of normally distributed populations and suitable statistical testing is required.

Response:
We have removed the baseline comparisons between the groups (Reviewer 1, point 8). The statistical reviewer felt that the use of mixed models analyses was appropriate.

10. On page 11 of the manuscript the authors suggest that removing a patient from the analysis did not change results. I am concerned that this has been considered as it would not be an analysis that would be supported i.e. to remove data that ' doesn't fit'.

Response:
The primary analyses included all participants in an intention-to-treat fashion. We conducted a sensitivity analysis to explore the effect of the results of the single participants with marked hypercalcaemia. We don't think this can be construed as suggesting we removed data that did not fit our hypothesis, but we would be happy to remove these analyses if the editor wishes.

11. Table 2 describes the various measures of calcium, vitamin D (various hydroxylated forms) and PTH in a single patient. In the first 4 weeks, when high dose supplementation was used, the levels of 25OHD increased with increased 1,25OHD (as expected as more substrate was present) with appropriate suppression of PTH in response to increasing serum calcium levels. By 6 weeks serum calcium levels rose to elevated, and clinically concerning, levels. On reducing the supplemental regimen to less frequent dosing, 25OHD, 1,25OHD and serum calcium fell to almost pre-treatment levels – with 25OHD levels remaining deficient, suggesting that the low dose regimen is unlikely to do much harm, but is also not demonstrably doing any good either. The presence of this figure is unhelpful and confusing and should be considered anecdote alone and should be removed. If data is present for all participants with appropriate standard deviations it may prove helpful.

Response:
The Reviewer has misunderstood the time sequence of events. The participant developed marked hypercalcaemia which led to the vitamin D supplementation being stopped. She did not take low dose supplements subsequently- this is stated clearly in the text "No further study medication was administered". Thus, the Reviewer's comments about the low dose regimen are incorrect.

We disagree with the Reviewer about the utility of this Table. It shows the time course of a severe case of hypercalcaemia, with a detailed profile of relevant variables. It highlights a number of important points- eg the low 25OHD/1,25OHD at baseline, and the time course of hypercalciuria, hypercalcaemia and laboratory abnormalities. We think this is an important table for the paper, and therefore have retained it in the text.

12. Figure 2 shows grouped data simply demonstrating that those given supplementation have higher levels than those who don't. It is of limited value to the clinical question asked but is the only positive finding.

Response:
The point of Figure 2 is that it shows clearly that average levels of serum 25OHD >75 nmol/L were obtained with vitamin D supplements. This is a level widely accepted as representing vitamin D sufficiency. Many previous trials of vitamin D supplements have been criticised because they did not achieve this level for the majority of participants.

13.Overall the paper in its current form does not allow me to draw any concrete conclusions with a number of methodological and conceptual issues to the study addressing what I'm not sure is a real clinical problem, other than that those receiving supplements have higher vitamin D levels than those that do not.

Response:
We disagree with the Reviewer. The paper shows one case of significant hypercalcaemia that raises concerns about the use of vitamin D supplements in sarcoidosis. In addition, the trial does not document any benefit on surrogate measures of skeletal health, suggesting that there are likely to be minimal benefits of vitamin D supplements, if any, for patients with sarcoidosis with vitamin D levels in the range studied. The results also do not support the establishment of large trials with fracture endpoints in higher risk populations.

Reviewer: 3
Comments to the Author
This study is a randomised controlled trial of Vitamin D supplementation in patients with sarcoidosis and low Vitamin D levels. The hypothesis and aims of the study should be stated more clearly. Most clinicians would consider Vitamin D supplementation in sarcoidosis in three situations:-

1. Oral corticosteroid use, to prevent low bone mineral density

2. Treat low bone mineral density

3. Low Vitamin D levels and persistent symptoms, such as lethargy

This study has not addressed any of these, and I am not sure how useful the primary outcome measure (serum calcium) is in this study.

Response:
We have clarified that the aim of the study was to "to determine the effects of vitamin D supplementation on surrogate measures of skeletal health in patients with sarcoidosis and vitamin D insufficiency." (Last sentence Introduction). We have discussed under Reviewer 1 Point 3 the reason for carrying out this trial in an intermediate risk population with osteopenia rather than a high risk population with low bone density. We think the results are likely applicable to higher risk populations and do not support the establishment of large trials with fracture endpoints in such populations. We have addressed the comment regarding serum calcium under Reviewer 2 point 1.

The safety data of Vitamin D supplementation from this study is very useful, the risk of hypercalcaemia was very low (4%). It could be detected at six weeks and all that was needed was Vitamin D withdrawal.

Response:

We agree with the Reviewer that the hypercalcaemia risk is valuable information. We think that the data are quite concerning. The patient was closely monitored in a clinical trial, and the hypercalcaemia was detected early when she was asymptomatic. We would caution against assuming that the same outcomes would occur in clinical practice where monitoring is not as intensive. These results suggest there is a small but real possibility of significant harm resulting from hypercalcaemia with the use of vitamin D supplements in sarcoidosis and therefore, if they are to be used, very close monitoring is required. The absence of evidence for a benefit of vitamin D supplements argues against their use in sarcoidosis.

VERSION 2 – REVIEW

REVIEWER	Jake Olivier School of Mathematics and Statistics University of New South Wales Sydney, Australia
REVIEW RETURNED	11-Sep-2013

- The reviewer completed the checklist but made no further comments.